and acidosis (pH 7.4 vs. 6.8 and 6.5) on myosin's ability to move actin. As expected, in the absence of added P_i, acidosis caused the actin filament velocity (V_{actin}) to progressively decrease from 6.1 ± 1.3 $\mu m \cdot s^{-1}$ (mean ± SD) at pH 7.4 to 1.6 ± 0.5 $\mu m \cdot s^{-1}$ at pH 6.5. Surprisingly, the addition of 30mM P_i caused V_{actin} to recover a significant amount of the acidosis-induced depression in V_{actin}, with the effect most pronounced at pH 6.5, (1.6 ± 0.5 vs 3.3 ± 1.3 $\mu m \cdot s^{-1}$, at 0 and 30mM P_i respectively). The effects of P_i were also dependent on the ATP concentration, with 30mM P_i slowing V_{actin} at low ATP levels (<500uM), but enhancing V_{actin} at high ATP levels (>2mM). The slowing of V_{actin} by P_i at low ATP was also pH dependent, being strongest at pH 7.4, reduced at pH 6.8, and completely reversed at pH 6.5. A simple detachment-limited kinetic model was constructed to explore the molecular basis of these observations. Good fits ($p>0.05$) to the data required that P_i be able to rebind to actomyosin in both the rigor (AM) and the ADP-bound (AM.ADP) states, prolonging the duration of strong actin binding (t_{on}) in the former case while decreasing t_{on} in the latter. Thus it appears that at the levels of acidosis and ATP experienced during fatigue, elevated P_i might enhance rather that inhibit myosin's ability to translocate actin under unloaded conditions.

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Force-Generating Capacity of Human Myosin Isoforms Extracted from Single Muscle Fiber Segments

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Muscle, motor unit and muscle fibre type-specific differences in force-generating capacity have been investigated for many years, but there is still no consensus regarding specific differences between slow- and fast- twitch muscles, which may relate to a number of different confounding factors disguising the myosin function, i.e. the molecular motor protein. The aim of this study is to evaluate the force-generating capacity of specific myosin isoforms extracted from human muscle fiber segments in a modified single fiber *in vitro* motility assay, in which an internal load (α-actinin, actin-binding protein) was added in different concentrations to inhibit force generation of the myosin. After the negative linear relationship between the fraction of moving filaments and the α-actinin concentration was plotted, both the slope and *x*-axis intercept were used as force index to evaluate force production. The force-generating capacity of the β/slow myosin isoform (type I) was weaker ($p<0.05$) than the fast myosin isoform (type II), but the force-generating capacity of the different fast myosin isoforms types IIa, IIx or IIax were inseparable. In conclusion, (i) a significant difference in force-generating capacity was observed between human slow and fast myosin isoforms; (ii) the modified single fiber *in vitro* motility assay presents a unique possibility to measure the force-generating capacity of specific myosin isoforms, and also provides a platform for studies on myosin function in the growing disease entity called "myosinopathies" as well as in the different post-translational modifications.

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The E706K IBM3 Myosin Mutation Depresses the Chemomechanical Properties and Increases the Lability of the Molecular Motor

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Hereditary myosin myopathies are muscle diseases with variable clinical features and age of onset. Inclusion body myopathy 3 (IBM3) is an autosomal dominant myopathy associated with a missense mutation (E706K) in the myosin heavy chain IIa gene (MYH2). The disease is mild in childhood but appears progressive in adulthood, with proximal muscle weakness affecting movement. Biopsies from adult patients reveal dystrophic alterations and rimmed vacuoles consistent with an increased expression of the mutant motor with advanced age. We constructed a transgene encoding E706K myosin and expressed it in Drosophila (E699K) indirect flight and jump muscles. Flight and jump abilities were reduced in heterozygous flies and were nearly absent in homozygotes consistent with the possible age-related dose-dependent response observed in patients. The mutant myosin displayed 80% lower actin sliding velocity and 74 and 83% reductions in basal and actin stimulated ATPase activities compared to wild-type myosin. Electron microscopy revealed E706K (E699K) myosin heads bear a dramatic propensity to collapse and to aggregate relative to wildtype heads. At 23°C, 77.5% (n=1146) of control molecules exhibited two well-resolved independent heads compared to only 22.5% (n=1014) of mutant myosin molecules. A five minute, 37°C incubation induced 80.9% (n=1181) of the control molecules' heads to form intra- or intermolecular aggregates versus 95.3% (n=1192) of the mutant myosin heads. This test directly assessed motor integrity and suggests E706K (E699K) myosin is far more labile than wildtype myosin. We are imaging mutant myocytes to determine if the ultrastructural hallmarks seen in adult patients also appear in our fly model. The depressed motor properties and the propensity for the mutant myosin to collapse and aggregate likely contribute to the muscle weakness observed in our fly model and possibly in senescent patients.

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Theoretical Analysis of the Stochastic Behaviour of Skeletal Actomyosin Complex

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The skeletal muscle models are usually based on the hypotheses proposed by AF Huxley [1,2], but only recently an experimental evidence of them has been obtained: in [3] a single Myosin II shows several 5.5 nm steps on an actin filament in one preferred direction per ATP cycle, in [4] the probability for the actomyosin complex to switch from a weakly to a strongly attached state increases when the myosin is stretched (SS).

We have computed the steady state solution of 2D Fokker-Plank equation associated to a system of a motor bound to a needle in the presence of an actin filament. We show how the dwell time of the stable states of the actomyosin complex is affected by the drag coefficient of the needle and use the data in [3] to estimate the bias of the energy toward the direction of the motion.

Based on this analysis, we have developed a mathematical model to analyse quantitatively the feasibility of the hypothesis proposed in [3]. The stepping behaviour is view as a diffusive process in a locally tilted but globally flat potential, while the SS affects the jump probability in the attachment-detachment process. The response of the model is analysed by a stochastic simulation of the associated Langevin equations and succeeds to reproduce the behaviour of the muscle in its short time scale, related to the power stroke, and in its long time scale, related to the actin-myosin attachment detachment cycle.

[1] Huxley. Prog Biophys Biophys Chem (1957)
[2] Huxley, Simmons Nature (1971)
[3] Kitamura et al. Biophysics (2005)
[4] Iwaki et al. Nat Chem Biol (2009)

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Conformational Changes in Myosin V Monitored by Hydrogen-Deuterium Exchange Mass Spectrometry

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Conformational changes between the apo and the BeF.ADP states of Myosin V were determined by hydrogen/deuterium exchange monitored by FT-ICR mass spectrometry.

In the BeF.ADP state we have observed opening of the 50K domain cleft (actin binding cleft) (aa 566-578) as expected from the x-ray structure (Coureux et al., Nature 2003). The opening of the actin binding cleft was coupled to the closure (decreased solvent accessibility) of the active site - a stretch of aa 197-231 that includes switch 1, loop1 and B6 β-strand and an additional peptide at the entrance to the active site (aa 233-237).

Unexpected changes were observed in the in the SH1 helix (aa 678-692), which was more exposed and the upper 50 kDa domain (aa 331-339 and 350-381) which was less exposed. More protection was observed in the loop coupling B3 β-strand to the SH1 helix (aa 662-676).

Figure: Conformational changes in Myosin V mapped on the crystal structure. Increased solvent accessibility in BeF.ADP in blue wide cylinders. Decreased accessibility in narrow red cylinders.

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Structural Dynamics of Myosin Investigated by Transient Time Resolved Fluorescence

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We are using transient time resolved fluorescence $(TR)^2F$ to map conformational transitions in the catalytic domain of *Dictyostelium* myosin II. The photophysical state of fluorescent probes, site specifically attached to proteins, shadows the underlying protein structure, dynamics and local electrochemical environment. Changes in these underlying constraints, affect the excited state lifetime of the probe. Because the lifetime decays exponentially, different